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Indomethacin and e.s.r. signals in uterine cervix

Tomasi *et al.* (1984) have reported e.s.r. spectroscopic studies of uterine cervixes of rats that had received intraperitoneal injections of indomethacin to inhibit the cyclo-oxygenase-dependent prostaglandin cascade. When a single dose of indomethacin was administered 3 days before sampling, it was found that at 77 K the anisotropic e.s.r. signal in the region of $g = 2.010$ was significantly lower than that which was observed with normal control samples. Two new and very intense signals with $g = 4.3$ and 2.24 were also reported for the samples from the indomethacin-treated animals, but were not commented on further in the paper, although they demonstrate that major changes in the chemical states of iron must be occurring.

The signals with $g = 4.3$ and 2.24 almost certainly both correspond to Fe(III), the former to the high-spin $S = 5/2$ state, with the latter possibly representing a low-spin, $S = 1/2$, form. Since they completely dominated the spectra of the uterine cervixes of indomethacin-treated animals, but were virtually absent from the spectra of normal control samples, we considered that they might represent an important consequence of the drug treatment. We have therefore attempted to repeat the experiments of Tomasi *et al.* (1984), using 60–70-day-old female Hooded Lister rats of the Rowett Institute strain fed on a standard laboratory diet (Labsure, from Lavender Mills, Manea, Cambs., U.K.) with free access to food. The reported experimental conditions were followed closely, i.e. animals (weighing 200–250 g) were given one intraperitoneal injection of indomethacin (Sigma, Poole, Dorset, U.K.) (10 mg/kg body wt., in 0.5 ml of 0.9% NaCl; controls received saline only) 3 days before being killed by cervical dislocation followed by exsanguination. Samples, which contained cervix, short lengths of upper vagina and lower uterus horns, were transferred immediately to liquid nitrogen without washing and then coarsely crushed under liquid nitrogen [Tomasi *et al.* (1984) report grinding their samples under liquid nitrogen]. However, no signals corresponding to Fe(III) were seen in any of our e.s.r. spectra. Nor were we able to produce these signals when the indomethacin was administered over a period of 3 days immediately before sampling or when aspirin (soluble aspirin; Nucros, Warminster, Wilts., U.K.) or Fenbufen (γ -oxo-1,1'-biphenyl-4-butanoic acid; from Lederle Laboratory, Division Cyanamid G.B. Ltd., Gosport, Hants., U.K.) was substituted for the indomethacin. Furthermore, although we generated an anisotropic free-radical signal with $g_{\perp} = 2.004$, $g_{\parallel} = 2.034$ in all samples, we did not find any statistically significant differences in intensity

between the control samples and those from the rats that had received the drug treatments.

It appears therefore that the results reported by Tomasi *et al.* (1984) are not a consistent consequence of the indomethacin treatment, but are the result of a combination of factors that we are unable to identify at present. It seems that the system is more complex than has been suggested, and further investigations are needed in order to establish the causes of the changes in free-radical signal and, perhaps more importantly, the dramatic changes in chemical forms of the iron reported by Tomasi *et al.* (1984).

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Indomethacin and e.s.r. signals in uterine cervix: a reply

We can reply as follows to the letter by McPhail *et al.*

Our paper (Tomasi *et al.*, 1984) was concerned mainly with the strong peroxy e.s.r. signal observed in normal uterine cervix after grinding the sample under liquid nitrogen. In a previous paper (Benedetto *et al.*, 1981) we had stressed the importance of grinding the frozen tissue in generating this peroxy signal; it is necessary to grind the tissue finely to obtain consistent results. We feel that a major reason for the failure of McPhail *et al.* to observe an effect of indomethacin on the peroxy signal was their use of coarsely crushed tissue fragments rather than a fine frozen powder; in our study, a significant decrease of about 60% was observed in the signal intensity.

With regard to the signal at $g \sim 4.3$, we showed an illustration of a treated sample giving a very strong signal, but the intensity at $g \sim 4.3$ was very variable from sample to sample, and could also be observed on occasion in untreated samples, but at much lower intensity.

The signal at around $g = 2.24$ was seen with high variability in normal human cervix samples (Benedetto *et al.*, 1981), but was not clearly evident in normal rat